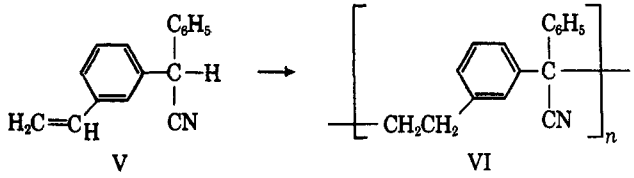

V VI

to styrene occurs only at the β-styrene position. Therefore the presence of methyl groups in the polymer from the *p*-styrene I must be due to the reaction of xylylene and not to the addition of the anion IV to the α-styrene position (route c_1).

Of the two remaining polymerization routes which can account for the structure IIIc in the polymer, the Michael addition (route c_2) appears to be more probable than the homopolymerization (route c_3). The ionization of the styrene I (R_1 = CN and R_2 = C_6H_5) to the anion IV was shown to be rapid and reversible. The formation of the anion can be noted visually by its red color and spectroscopically by its absorption at 485 mμ. Studies of proton exchange with deuterium showed that the acetonitrile proton is completely equilibrated in 1 *N* methanolic potassium hydroxide within 15 min. at room temperature. On the other hand, after 16 hr. of equilibration in the presence of deuterium ions no evidence was obtained for the addition of deuterium to the vinyl group. (Under these conditions most of the starting styrene was recovered unpolymerized.) This indicates that the addition of a proton to the *p*-quinoid resonance form of IV to give the xylylene is a slow process and perhaps, due to rapid reaction of the xylylene, not reversible. Because of its slow rate of formation and high reactivity the xylylene must be present in solution in extremely low concentration. Under such conditions and in the presence of large amounts of the anion IV (or a polymeric material containing this anionic structure) the Michael addition of IV to II (route c_2) appears to be the more probable reaction course. The head-to-tail arrangement of the structure IIIc in the polymer is in accord with such a mechanism although it does not rule out the homopolymerization (route c_3).

The present experiments demonstrate the presence of an equilibrium between a styrene and a xylylene but they do not measure the equilibrium constant. The ratio of IIIb to IIIc observed in the polymer is kinetically controlled and only indirectly dependent on the equilibrium concentration of the xylylene. The large amount of IIIc observed can be rationalized on the grounds that the rate of addition of the anion IV to the styrene I is slow (possibly due to steric effects around the anion) and the rate of formation of the xylylene II is sufficiently favored (electronic effects) to account for the observed ratio.

An alternate system of interest is that of 9-vinylanthracene (resonance energy 5.75β) and 9,10-dihydro-9,10-dimethyleneanthracene (resonance energy 5.68-β).[3,14] The small difference in energy between these compounds should allow ready interconversion in a suitably substituted system. However, an initial attempt to synthesize 9-vinyl-10-cyanomethylanthracene was not successful.

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Synthesis of 2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactose

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The synthesis of 3-*O*-β-galactosyl-*N*-acetylgalactosamine (V) is reported. It involves condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (IX), either with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (II), or with compound VI. In the latter case advantage is taken of the differential reactivity of the hydroxyl groups in the galactopyranose ring.

The disaccharide 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactose (V) was isolated from an acid hydrolysate of human blood group A substance.[1] It is also found in hydrolysates of blood group B, H, and Le[a] substances[2] and forms an essential part of their antigenic determinants.[3] Recent studies have shown that this disaccharide is inherent in the structure of the brain gangliosides[4,5] in which it constitutes the terminal part of the molecule.[6,7] The chemical[8] and enzymatic[9,10] syntheses of the glucosamine analog of V, also occurring in the blood group substances, have recently been reported. The present synthesis was undertaken in connection with a study on galactosamine-containing oligosaccharides of *N*-acylsphingosine.

Benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside (I) was prepared from 2-acetamido-2-deoxy-α-D-galactose by treatment with a solution of gaseous hydrogen chloride in benzyl alcohol at 70°. Although the corresponding glucosamine derivative has been prepared at reflux temperature,[11] these conditions caused extensive decomposition here, and only a negligible yield of the desired glycoside could be isolated. The reaction at 70° was followed polarimetrically and a maximum rotation was obtained after 8–10 hr. The crude amorphous product, isolated by precipitation with ether, was probably contaminated with a little of the β-isomer

(1) T. J. Painter, I. A. F. L. Cheese, and W. T. J. Morgan, *Chem. Ind.* (London), 1535 (1962).
(2) V. P. Rege, T. J. Painter, W. M. Watkins, and W. T. J. Morgan, *Nature*, **200**, 532 (1963).
(3) T. J. Painter, V. P. Rege, and W. T. J. Morgan, *ibid.*, **199**, 569 (1963).
(4) E. Klenk, U. W. Hendricks, and W. Gielen, *Z. Physiol. Chem.*, **330**, 140 (1962).
(5) R. Kuhn and H. Wiegandt, *Ber.*, **96**, 866 (1963).
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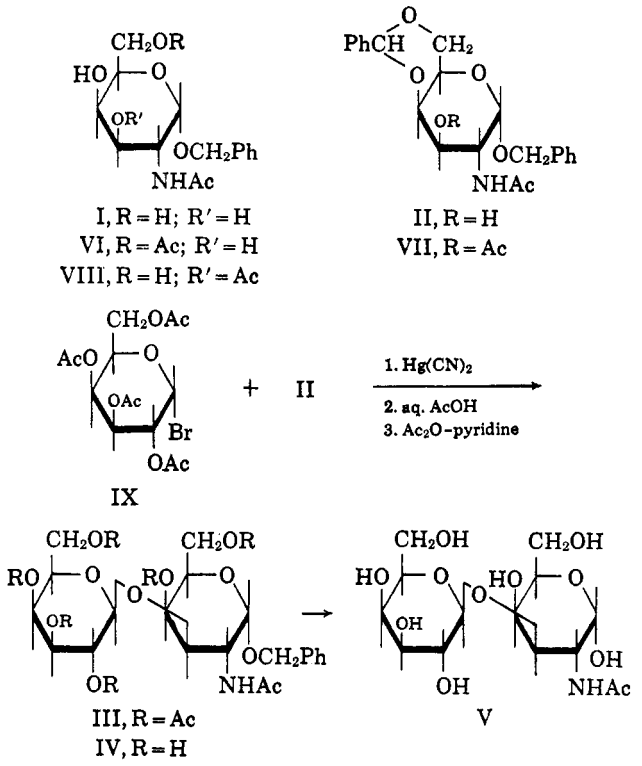
(9) A. Alessandrini, E. Smidt, F. Zilliken, and P. Gyorgy, *J. Biol. Chem.*, **220**, 71 (1956).
(10) M. C. Glick, I. W. Chen, and F. Zilliken, *ibid.*, **237**, 981 (1962).
(11) R. Kuhn, H. H. Baer, and A. Seeliger, *Ann.*, **611**, 236 (1958).

and could not be induced to crystallize. However, it was readily converted to the pure, crystalline 4,6-*O*-benzylidene derivative (II) in good yield. The pure glycoside I could be obtained from the latter compound after removal of the benzylidene group. Its extremely high positive rotation is typical of an α-D-isomer.



Condensation of II with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (IX) under conditions similar to those previously described[8] gave a mixture which could not be separated satisfactorily. However, when the crude reaction product was treated with acetic acid to remove the benzylidene group and then acetylated, the hexaacetate (III) could be obtained as a pure, crystalline solid after chromatography. Catalytic saponification[12] of III gave IV in high yield. This was hydrogenolyzed in alcoholic solution containing a little acetic acid, in the presence of palladium, to give the disaccharide V. This compound showed complete identity in melting point, optical rotation, infrared spectrum, and thin layer chromatography with the natural product.[4] The low optical rotation, method of synthesis, and infrared spectrum indicate a β-configuration for the disaccharide linkage.

An alternative route leading to V takes advantage of the expected differential reactivity of the primary hydroxyl and the hydroxyls at C-3 and C-4 in I. The latter group, being axially oriented in the stable C-1 conformation, is of lowest reactivity.[13] In fact, the primary hydroxyl function of I could be selectively acetylated to give VI, a compound which was different from the isomeric 3-acetate VIII obtained by acetylation of II and removal of the benzylidene group from the resulting compound VII. Reaction of VI with IX, followed by acetylation, led to the isolation in good yield of a disaccharide identical with III.

(12) G. Zemplen, *J. Am. Chem. Soc.*, **52**, 2568 (1930).

(13) R. J. Ferrier and W. G. Overend, *Quart. Rev.* (London), **13**, 265 (1959).

Experimental[14]

Benzyl 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-galactopyranoside (II).—One gram of *N*-acetylgalactosamine (m.p. 163–164°) was stirred overnight at 70° with 12 ml. of a 2% solution of gaseous hydrogen chloride in benzyl alcohol. Excess dry ether was added to the cooled solution and the amorphous precipitate was removed by filtration, washed thoroughly with dry ether, and dried, giving 1.1 g. of m.p. 186–190°, $[\alpha]^{20}D$ +190° (*c* 1.0, water). This material was shaken for 24 hr. with 4 ml. of freshly distilled benzaldehyde and 1.0 g. of fused zinc chloride. The clear solution obtained was shaken with an excess of cold water and *n*-pentane, and the white precipitate produced was separated by filtration, washed thoroughly with water and pentane, and dried. Crystallization from 60% pyridine in water gave 0.75 g. (40%) of needles, m.p. 243–245°. Recrystallization from the same solvent mixture raised the melting point to 246–247°, $[\alpha]^{28}D$ +219° (*c* 2.12, pyridine).

Anal. Calcd. for $C_{22}H_{25}NO_6 \cdot H_2O$: C, 63.35; H, 6.52. Found: C, 63.23; H, 6.52.

The water of crystallization was held very tenaciously and could not be removed even at 80° *in vacuo*.

Benzyl 2-Acetamido-2-deoxy-α-D-galactopyranoside (I).—A solution of 700 mg. of II in 5 ml. of 60% acetic acid was heated for 30 min. at 100°. The acetic acid was removed *in vacuo* by coevaporation with several portions of water to prevent undue concentration of the acid. The dried residue was dissolved in hot methanol, and the addition of a little dry ether precipitated 500 mg. (95%) of needles, m.p. 202–204°. Recrystallization from methanol containing a little ether gave a product of m.p. 203–205°, $[\alpha]^{23}D$ +204° (*c* 0.98, water).

Anal. Calcd. for $C_{15}H_{21}NO_6$: C, 57.86; H, 6.80; N, 4.50. Found: C, 57.91; H, 6.72; N, 4.41.

Benzyl 2-Acetamido-6-*O*-acetyl-2-deoxy-α-D-galactopyranoside (VI).—I (500 mg.), m.p. 202–204°, was dissolved in 2 ml. of dry pyridine and the solution was cooled to 0°. After addition of acetic anhydride (0.16 ml., 0.98 mole equiv.), the solution was allowed to warm slowly and left at 20° for 1 hr. To the cooled mixture, excess methanol was added, and the solution was evaporated *in vacuo*. The residue was dissolved in ethylene dichloride and purified by chromatography on silica gel. A mixture of ethyl acetate and acetone (3:1) eluted 350 mg. (61%) which crystallized in needles from acetone containing a little methanol, giving 200 mg. of m.p. 196–198°, $[\alpha]^{20}D$ +188.8° (*c* 1.08, methanol); the melting point was unaffected by recrystallization. Acetone eluted 125 mg. (22%) of unreacted I. (No di-*O*- or tri-*O*-acetylated material could be isolated under these conditions.)

Anal. Calcd. for $C_{17}H_{23}NO_7$: C, 57.78; H, 6.55; N, 4.00; total acetyl, 24.3. Found: C, 57.79; H, 6.61; N, 4.20; total acetyl, 23.4.

An identical product was obtained directly from crude benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside of m.p. 186–190°, in 33% yield.

Benzyl 2-Acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-α-D-galactopyranoside (VII).—II (100 mg.) was acetylated in the usual manner with 0.5 ml. of acetic anhydride in 0.5 ml. of pyridine. Evaporation *in vacuo* left a sirup which was dissolved in benzene and purified by chromatography on silica gel. Ether eluted fractions totalling 90 mg. Crystallization from acetone containing a little ether gave prisms of m.p. 160–162°, with previous sintering at 93° and resolidifying on further heating. After a second recrystallization from the same solvent mixture, the

(14) Melting points were taken between glass slides on a Fisher-Johns apparatus and were corrected. Rotations were determined in semimicro tubes using a Zeiss Kreis polarimeter, 0.01°; the chloroform used was dry and free of ethanol. Infrared spectra were determined on an Infracord spectrometer. Chromatograms were made with the flowing method using silica gel (Silica Gel Davison, grade 950, 60–200 mesh) without pretreatment. The elution was stepwise, in order of increasing polarity of the solvents. The proportion of weight of substance to be adsorbed to weight of absorbent was 1 to 50–100. The fractions eluted were 2 ml./g. wt. of the column. Thin layer chromatograms were made using kieselgel G (E. Merck, Darmstadt). The microanalyses were performed under the direction of R. Heller of the Weizmann Institute of Science.

product melted at 161–163°, with sintering at 95°, $[\alpha]^{25}D$ +164° (*c* 1.00, chloroform).

Anal. Calcd. for $C_{24}H_{27}NO_7$: C, 65.29; H, 6.17; N, 3.17. Found: C, 65.45; H, 6.18; N, 3.20.

Infrared spectrum in $CHCl_3$ showed absorptions of equal intensity at 5.80 and 5.96 μ due to acetate and amide groups, respectively.

Benzyl 2-Acetamido-3-*O*-acetyl-2-deoxy-α-D-galactopyranoside (VIII).—II (150 mg.) was acetylated as described for VII; the residue obtained on evaporation *in vacuo* was heated for 30 min. with 60% acetic acid at 100°. After removal of the acid by coevaporation with water, the dry residue was dissolved in ethyl acetate and purified by chromatography on silica gel. A mixture of ethyl acetate and acetone (3:1) eluted 120 mg. (93%). Crystallization from a mixture of acetone and ether gave needles of m.p. 161–162° which was unaffected by recrystallization, $[\alpha]^{24}D$ +144° (*c* 1.04, chloroform).

Anal. Calcd. for $C_{17}H_{23}NO_7$: C, 57.78; H, 6.55. Found: C, 57.71; H, 6.37.

Infrared spectrum in $CHCl_3$ showed OH at 2.90 and bands of approximately equal intensity at 5.70 (acetate) and 5.96 μ (amide).

Benzyl 2-Acetamido-4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside (III). A. From II.—A solution was prepared containing 0.90 g. of II in a mixture of 30 ml. of nitromethane and 30 ml. of benzene, and moisture was removed by azeotropic distillation of 10–15 ml. of the solvent. After cooling to 25°, 0.56 g. (1 mole equiv.) of mercuric cyanide and 0.93 g. (1 mole equiv.) of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (IX) were added, and the solution was stirred (with exclusion of moisture) at 25° for 24 hr. A further 0.40 g. of mercuric cyanide and 0.68 g. of IX were then added, and the reaction was continued for 24 hr. more. After filtration, the clear solution obtained was diluted with benzene and washed several times with a saturated solution of sodium bicarbonate, then with water, dried, and evaporated, *in vacuo*. The residual sirup was heated with 5 ml. of 60% acetic acid solution at 100° for 30 min., the solvent was evaporated, and the residue was dried thoroughly over phosphorus pentoxide. Acetylation was effected with acetic anhydride and pyridine, and the sirup obtained, after evaporation *in vacuo*, was dissolved in benzene and purified by chromatography on silica gel. Ether eluted fractions totalling 1.07 g. Crystallization from a mixture of acetone and ether gave 0.55 g. of needles (34%), m.p. 160–162° which was unaffected by recrystallization, $[\alpha]^{26}D$ +80° (*c* 0.97, chloroform).

Anal. Calcd. for $C_{33}H_{43}NO_{17}\cdot H_2O$: C, 53.29; H, 6.10. Found: C, 53.40, 53.25, 53.28; H, 6.06, 5.95, 5.97.

The analyses were performed on samples from three separate preparations which were dried at 80° *in vacuo* for 24 hr.

B. From VI.—VI (230 mg.) was allowed to react with molar equivalents of IX and mercuric cyanide in 10 ml. of nitromethane at 35° for 24 hr. Further portions of IX and of mercuric cyanide (0.6 mole equiv.) were added, and the reaction was prolonged for a further 24 hr. The solution was filtered and evaporated *in vacuo*. Acetylation of the residual sirup with acetic anhydride in pyridine gave a product which was dissolved in benzene and purified by chromatography on silica gel. Ether eluted fractions totalling 0.25 g. (53%). Recrystallization from acetone and ether gave 150 mg. (31%) of m.p. 159–161°, $[\alpha]^{25}D$ +82° (*c* 1.00, chloroform). The melting point was not depressed by the product obtained from II. Both glycosides showed identical infrared spectra and identical thin layer chromatograms using 5% methanol in benzene as developing solvent (R_x 1.7, where x is 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-α-D-glucopyranose).

Benzyl 2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactopyranoside (IV).—A solution of 0.50 g. of III in 5 ml. of dry methanol, in which a catalytic amount of sodium had been dissolved, was allowed to stand overnight at 20°. A gel was produced which dissolved on warming. Scratching induced crystallization in needles. The yield was 0.26 g. (81%), m.p. 251–253°. Recrystallization from methanol raised the melting point to 252–254°, $[\alpha]^{25}D$ +108° (*c* 0.87, 95% ethanol).

Anal. Calcd. for $C_{21}H_{31}NO_{11}\cdot H_2O$: C, 51.29; H, 6.77; N, 2.85. Found: C, 50.92; H, 6.52; N, 2.95.

The substance was pure as shown by thin layer chromatography on silica gel using methanol, acetic acid, and water (4:5:1) as developing solvent, and *p*-anisaldehyde–sulfuric acid to reveal the spots; $R_{lactose}$ 2.0.

2-Acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactose (V).—A solution of 100 mg. of IV in 50 ml. of 95% alcohol, containing 1 ml. of acetic acid, was shaken at room temperature for 24 hr. with 50 mg. of 10% palladium on charcoal and hydrogen at 45 p.s.i. After filtration, the clear solution was evaporated *in vacuo* to a white solid which crystallized from alcohol in fine needles, weighing 50 mg., m.p. 157–159° dec. After recrystallization from alcohol it melted at 159° dec. The substance was hygroscopic, and it was difficult to remove the mole of water of crystallization present; $[\alpha]^{25}D$ +52.0° (5 min.) → +31.0° (equilibrium, *c* 1.0, water).

Anal. Calcd. for $C_{14}H_{25}NO_{11}\cdot H_2O$: C, 41.86; H, 6.78; N, 3.49. Found: C, 41.69; H, 6.61; N, 3.25.

Anal. Calcd. for $C_{14}H_{25}NO_{11}$: C, 43.85; H, 6.57. Found (after drying for 24 hr. at 110° under high vacuum): C, 43.78; H, 6.64.

Infrared spectrum in KBr showed bands at 3.0 (OH), 6.1 and 6.45 (amide), 11.20 (β-disaccharide), and 11.45 μ (galactopyranoside ring). Thin layer chromatography using 1-butanol–acetone–water (4:5:1) showed $R_{lactose}$ 1.1.

Acknowledgment.—We wish to thank Professor E. Klenk for the gift of a sample of natural 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-α-D-galactose.